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REMARKS

Claims 11, 15, 16, and 21-26 are now of record in the case. Claims 11, 16, 21, 22, and 25 have been amended, claims 12-14 and 17-20 have been cancelled, and new claim 26 has been added. Non-elected claims 1-10 were cancelled previously.

Support for the amendments to the claims is inherent in the original disclosure. Specifically, with respect to the additional components recited in claim 1, support for the termite aggregation attractant may be found in original claim 16. Support for the recitation of a termite pheromone may be found in previously presented claim 25 and in the specification at paragraph no. 0031 on page 11 of the specification. Support for the recitation of a bait matrix comprising a cellulose-containing material, may be found in original claim 21 and previously presented claim 22, and in the specification at paragraph no. 0029 bridging pages 9 and 10. The recitation in claim 1 that the *Paecilomyces* comprises spores may be found in original claim 14 and paragraph no. 0027 bridging pages 8 and 9 of the specification. The recitation in claim 1 that the *Paecilomyces* comprises *P. fumosoroseus* or *P. javanicus* may be found in original claims 12 and 13, and in the specification at paragraph no. 0024 bridging pages 7 and 8. Support for the amendment to claim 21 that the bait matrix comprises a cellulose containing material may be found in previously presented claim 22, and in

the specification at lines 12-14 of paragraph no. 0029 bridging pages 9 and 10. The recitation of the various cellulose-containing materials recited in claim 22 is supported by the specification at lines 13-20 of paragraph no. 0029 bridging pages 9 and 10. Finally, support for new claim 26 reciting that the bait matrix may be a solid, semi-solid, or liquid may be found at lines 3-5 of paragraph no. 0029 bridging pages 9 and 10.

Rejection Under 35 U.S.C. §102

Claims 11, 12, 14, 15, and 17-21 have been rejected under 35 U.S.C. 102(b) as being anticipated by Osborne. Applicants respectfully disagree and have amended the claims in an effort to more clearly differentiate over the reference.

Osborne discloses methods and compositions for controlling whiteflies using the fungus *Paecilomyces fumosoroseus*. Spores of the fungus are formulated in water with a wetting agent and an optional powder or granular carrier (col. 4, line 63 to col. 5, line 30.

The instant invention is drawn to compositions for control of termites. Applicants have discovered that strains of the entomopathogenic fungus of the genus *Paecilomyces* are useful for control of infestations by subterranean termites. Spores of *P. fumosoroseus* or the closely related *P. javanicus* are used in a preferred embodiment (paragraph nos. 0024 and 0027). In another

preferred embodiment, the fungus is formulated in a bait matrix which contains a form of cellulose (paragraph no. 0029). The fungus may also be formulated with optional attractants such as a termite pheromone or a termite aggregation attractant (paragraph no. 0031). This is not disclosed or suggested by Osborne.

Although Osborne does disclose using *Paecilomyces* as a biocontrol agent, the patent discloses its use against an insect, the whitefly, which is entirely unrelated to the termites disclosed and claimed in the instant invention. Moreover, because the insects are unrelated, a practitioner of ordinary skill in the art would have no motivation to use the *Paecilomyces* of Osborne against termites as claimed.

In an effort to more clearly differentiate the instant invention, applicants have amended claim 1 to limit the claimed composition to additional components which are particularly preferred for use against termites. Specifically, claim 1 has been limited to a component selected from one or more of a termite aggregation attractant, a termite pheromone, and a bait matrix comprising a cellulose-containing material. None of these components are disclosed or suggested by Osborne. Moreover, a practitioner of ordinary skill in the art would have no motivation to use any of these components, which are suited for controlling termites, in a composition for controlling whiteflies such as disclosed by Osborne.

Rejection Under 35 U.S.C. §102

Claims 11, 12, 14, 15, and 17-19 have been rejected under 35 U.S.C. 102(b) as being anticipated by Jackson. Applicants respectfully disagree and have amended the claims in an effort to more clearly differentiate over the reference.

Jackson discloses a novel technique for producing spores of *Paecilomyces fumosoroseus* which may be subsequently used as a biocontrol agent against insects such as the whitefly. The patent discloses that the spores may combined with lactose and bovine serum albumin and dried for storage (col. 11, lines 26-30), and may be formulated with water and diatomaceous earth for application against whiteflies (col. 11, lines 50-55).

As with Osborne, Jackson does not disclose or suggest using the *Paecilomyces* against termites, but rather against the whitefly, which is entirely unrelated to the termites disclosed and claimed in the instant invention. Again, because the insects are unrelated, a practitioner of ordinary skill in the art would have no motivation to use the *Paecilomyces* of Jackson against termites as claimed.

The amendments to claim 1 which were discussed in the rejection over Osborne, similarly further distinguish over Jackson. As above, the amendments limit the claimed composition to additional components which are particularly preferred for use against termites, with claim 1 being limited to a component

selected from one or more of a termite aggregation attractant, a termite pheromone, and a bait matrix comprising a cellulose-containing material. None of these components are disclosed or suggested by Jackson, and a practitioner of ordinary skill in the art would have no motivation to use any of these components, which are suited for controlling termites, in a composition for controlling whiteflies such as disclosed by Jackson.

The Rejection Under 35 U.S.C. §102(b)

Claims 11, 14-17, 21, 22, and 25 have been rejected under 35 U.S.C. §102(b) as being anticipated by Gunner *et al*. Applicants respectfully disagree and have amended the claims in an effort to more clearly differentiate over the reference.

Gunner *et al*. relates to the biological control of termites using an entomopathogenic fungus, such as *Metarhizium anisopliae* or *Beauveria bassiana* in combination with a behavioral modifier. The behavioral modifier may be a fungus that attracts termites, such as *Gloeophyllum trabeum* or its volatile products. Alternatively, the modifier may be a fungus that is repellent to termites, such as *Metarhizium anisopliae*. The **only** mention of *Paecilomyces* in the disclosure is in Claims 10 and 18, wherein it is described as being an option for the entomopathogen; and in Claims 26 and 33, wherein it is described as being an option for a fungus that is both entomopathogenic and repellent. Nowhere in

the disclosure does Gunner mention either *P. fumosoroseus* or *P. javanicus*, nor is there any disclosure outside of the claims how the *Paecilomyces* should be formulated. There is no teaching or suggestion that either of these species would be entomopathogenic and/or repellent.

The instant invention was described *supra*. As amended, the claims are now limited to compositions of spores of *Paecilomyces fumosoroseus* or *P. javanicus*. Neither of these species are disclosed or suggested by Gunner. Moreover, Gunner does not disclose or suggest using spores of the fungi.

The NCBI database at:

http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi

lists 18 named species of *Paecilomyces*, one unnamed species, and 16 isolates. This constitutes a total of 35 possibilities, of which *P. fumosoroseus* and *P. javanicus* are two. Given this vast array of choices, the person of ordinary skill in the art confronted with the teachings of Gunner *et al.* would not have sufficient direction or motivation to select the claimed species.

Moreover, Khader Khan *et al.* (1993, Insect Sci. Applic., 14:529-535, a copy of which is enclosed herewith), which was cited in the parent application, would further teach away from the instant invention. Specifically, Khader Khan teaches in Table 1 and at the top of page 532 that *Paecilomyces fumosoroseus* is slightly pathogenic against *Odontotermes obesus* (Rambur), an

African and South Asian termite. *O. obesus* belongs to the family Termitidae, a completely different family from the Rhinotermitidae described in Applicants' disclosure and recited in the claims presented for examination. Table 2 of the reference shows that the mortality rate of *O. obesus* treated with *P. fumosoroseus* was a mere 2% for workers major and workers minor, and 1% for soldiers. Of the nine fungal pathogens evaluated by Khader Khan, *P. fumosoroseus* was by far the worst, significantly inferior to the mortality rates as high as 80% observed for the other fungal pathogens evaluated. Based on these results, there would be absolutely no motivation for the person of ordinary skill in the art to even consider using *P. fumosoroseus* as a fungal control agent against other species of termites, particularly those belonging to a different taxonomic family than *O. obesus*. If anything, the disclosure of Khader Khan teaches away from the invention.

In contrast to the results observed by Khader Khan, Applicants demonstrate a mortality rate of 75-100% after 20 days post-exposure for the several strains of *P. fumosoroseus* evaluated in Example 1 against the Formosan subterranean termite (FST; *Coptermes formosanus* Shiraki; see FIG. 1). Two of the strains tested in Applicants' Example 1 gave a mortality rate of 100% within 6 days at an application rate of 10^9 blastospores/ml. Comparable, or even better results were reported in Applicants'

Examples 2-5 (FIGS. 2-5) for various strains of *P. fumosoroseus* against either FST or native subterranean termites (*Reticulitermes flavipes*). Example 6 shows mortality rates approaching 80% for native subterranean termites exposed to *P. javanicus*. In Example 7, *P. javanicus* achieves 100% mortality of Formosan subterranean termites in 13 days post-exposure.

Thus, the skilled artisan would be led away from using *P. fumosoroseus* in view of the negative results reported for that species as a control agent for *Odontotermes obesus*.

Rejection Under 35 U.S.C. §102(b)

Claims 11, 14-17, 21, 22, and 25 have been rejected under 35 U.S.C. §102(b) as being anticipated by Stamets. Applicants respectfully disagree and have amended the claims in an effort to more clearly differentiate over the reference.

Stamets discloses using the pre-sporulation (precondial) mycelial stage of various entomopathogenic fungi, including *Paecilomyces* spp., for the control of a variety of insects, including termites. At numerous instances in the patent, Stamets expressly teaches the criticality of using mycelia of the fungi which are at a preconidial stage of growth which is prior to conidia or spore formation. See, for example, lines 1-5 of paragraph no. 0018 on page 2, paragraph no. 0022 on page 3, and paragraph no. 0030 on page 3.

The instant invention was described *supra*. Again, the instant claims have been limited to spores of the fungus, in accordance with the preferred embodiment of the instant invention, wherein spores of *P. fumosoroseus* or the closely related *P. javanicus* are used for control of termites (paragraph nos. 0024 and 0027). This is not disclosed or suggested by Stamets.

Stamets expressly discloses using mycelia of the fungus at a preconidial stage, before conidia or spores are formed (lines 1-5 of paragraph no. 0018 on page 2, paragraph no. 0022 on page 3, and paragraph no. 0030 on page 3). Thus, the reference would actually teach away from using spores of the fungus against termites. Indeed, the reference would also teach against using spores of any of the fungi of the other references against termites.

As noted *supra*, a copy of the publication of Khader Khan *et al*. has been enclosed for the Examiner's convenience. The document has not been submitted with an Information Disclosure Statement (with its accompanying fees) or listed on a form PTO-1449 because it does not: (1) establish a *prima facie* case of unpatentability, or (2) refute any position taken by applicants, as defined by 37 CFR 1.56(b).

In view of the foregoing, applicants respectfully submit that claims 11, 15, 16, and 21-26 distinguish over the prior art of record. Allowance thereof is respectfully requested.

Respectfully submitted,

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enclosure:
--Khader Khan *et al.* (1993, Insect Sci. Applic., 14:529-535 (7 pages)

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MUSCARDINE FUNGI FOR THE BIOLOGICAL CONTROL OF AGROFORESTRY TERMITE *ODONTOTERMES OBESUS* (RAMBUR)

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Abstract—Among the eight entomopathogenic fungi tested against the termite, *Odontotermes obesus* (Rambur), five fungal pathogens viz., *Beauveria bassiana* (Bals.) Vuill, *Metarhizium anisopliae* (Metsch.) Sorokin var. *anisopliae*, *M. flavoviride* Gams Rozsypal var. *minus*, *Paecilomyces lilacinus* (Thom) Samson and *P. fumosoroseus* (Wize) Brown & Smith, were pathogenic. Three other fungal pathogens viz. *Verticillium lecanii* Zimm, *Paecilomyces farinosus* (Holm. ex Gray) and *Nomuraea rileyi* (Farlow) were not pathogenic. *B. bassiana* was the most effective pathogen against termites, followed by *M. anisopliae*, *M. flavoviride*, *P. lilacinus* and *P. fumosoroseus*. Workers minor, of the three morphogenetic forms of *O. obesus*, were the most susceptible, followed by workers major and soldier caste. Bioassay on isolates of two most virulent termite pathogens, *B. bassiana* and *M. anisopliae* revealed that *B. bassiana* isolate Bapatla was the most effective fungal pathogen with the lowest LC_{50} (9.98×10^{6} conidia/ml)

Key Words: Odontotermes obesus, bioassay, fungal pathogens

Résumé—Huit champignons entomopathogènes ont été testes contre les termites *Odontotermes obesus* (Rambur). Cinq espèces se sont révélées efficaces: *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorokin var. *anisopliae*, *M. flavoviride* Gams Rozsypal var. *minus*, *Paecilomyces lilacinus* (Thom.) Samson et *P. fumosoroseus* (Wize) Brown et Smith. *B. bassiana* a été le plus efficace suivi par *M. anisopliae*, *M. flavoviride*, *P. lilacinus* et *P. fumosoroseus*. Sur les trois morphotypes de *O. obesus* testés, les petits ouvriers ont été les plus effectés, suivis par les grands ouvriers puis les soldats. Les trois autres champignons pathogènes, *Verticillium lecanii* Zimm., *Paecilomyces farinosus* (Holm. ex Gray) et *Nomureae rileyi* (Farlow) n'ont pas donné de resultat positif. Les essais biologiques sul les isolats obtenus sur les deux entomopathogènes les plus virulents, *B. bassiana* et *M. anisopliae*, ont montre que l'isolat *B. bassiana* Bapatla a été le pathogène le plus efficace avec les valeurs LC_{50} (9.98×10^{6} conidia/ml) et LT_{50} (83,66hr) les plus faibles.

Mots Clés: Odontotermes obesus, essai biologique, champignon pathogène

INTRODUCTION

Termites are an important pest of agriculture, horticulture and wood work of buildings and they are highly organized social insects. Their hidden way of life and difficulties in exactly locating the sub soil nests of subterranean termites make a direct chemical control extremely difficult.

Search for alternatives to chemical pest control has assumed greater importance in recent years, due to the increasing cost of synthetic chemical pesticides, outbreak of secondary pests, insecticidal resistance and pollution problems. One such alternate method is biological control. Entomopathogenic fungi have been used in the past to control many pest species of economic importance, (Jayaraj, 1985, 1986). Except for the report made by Hanel (1981) no quantitative

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bioassays have been developed to compare the virulence of fungal pathogens agai[illegible]ermites. Although Hanel (1982) and Hanel and Wa[illegible]n (1983) did get some encouraging results, the biological control of *Nusutitermes exitiosus* (Hill) with *Metarhizium anisopliae* Sorokin in Australia was not a permanent one. In India, except for the reports by Sannasi (1969) that *Aspergillus flavus* Link ex Gray was pathogenic to termite queen, practically no work has been done with fungal pathogens. Hence, the present study was conducted under laboratory conditions to establish the pathogenicity of different fungal pathogens and compare their virulence against termites.

MATERIALS AND METHODS

Source of termites

Termites were cultured in the laboratory on soil, soft wood and fungus garden collected from the termitarium as per Ausa et al. (1962). The cultures were maintained at 25 ± 2°C and 96–100% r.h. The fungal combs with termites were taken and were carefully broken on a smooth surface, and the different termite castes were separated using a fine hair brush. The three morphogenetic forms could easily be recognized (Agarwal, 1978). The workers were food gatherers and formed the bulk of the termite

Table 1. Different fungal isolates, their source and culture medium used

Fungal isolate	Source	Fungus further referred	Mycological culture medium used
Beauveria bassiana	International Rice Research Institue (IRRI), The Philippines	PHP	Sabouraud dextrose agar enriched with 1% yeast extract (SDAY)
	Indian Agricultural Research Institute (IARI), New Delhi	NDL	SDAY
	Indian Institute of Horticultural Research (IIHR) Bangalore, Karnataka	BNG	SDAY
	Anamalai Hills, Coimbatore Tamil Nadu	CBE	SDAY
	Agricultural Research Station Bapatla, Andhra Pradesh	BPT	SDAY
Metarhizium anisopliae	IRRI, The Philippines	Ma	Emerson's yeast phosphate soluble starch agar (YPS agar)
M. flavoviride	IRRI, The Philippines		(YPS agar)
Paecilomyces lilacinus	IIHR, Bangalore, Karnataka	–	Potato dextrose agar (PDA)
P. fumosoroseus	Kerala Forest Research Institute (KFRI), Peechi, Kerala	–	PDA
P. farinosus	Kerala Forest Research Institute (KFRI), Peechi, Kerala		PDA
Nomuraea rileyi	IRRI, The Philippines	–	YPSs agar
Verticillium lecanii	Tamil Nadu Agricultural University (TNAU) Coimbatore, Tamil Nadu	–	PDA

PHP = Phillipines isolate.
NDL = New Delhi isolate.
BNG = Bangalore isolate.
CBE = Coimbatore isolate.
BPT = Bapatla isolate.

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Screening assays

The fungal isolates obtained from different sources were grown on standard culture media as given in Table 1. The isolates which had grown for 10 days at 25°C were used for the preparation of the inoculum. The conidia were harvested freshly just before application by washing from the surface of the culture plates using 75 ml solution of 0.02% Tween 80[R] (Rembach et al., 1986).

The harvested conidial suspensions were filtered through double layer muslin and centrifuged (3000 rpm) for 20 min and then resuspended in 25 ml solution of 0.02% Tween 80[R]. Conidial concentration in the filtrates was determined with the help of an improved Neubauer haemocytometer and conidial viability was determined as suggested by Gillespie (1986). Different concentrations of inoculum ranging from 10^3 through 10^8 conidia/ml were standardized by dilutions using sterile distilled water containing 0.02% Tween 80[R] (Roberts and Yendol, 1971).

Pathogenicity tests with different fungal isolates were conducted using a uniform dose of 10^7 conidia/ml in water. The termites were taken in a Petri-dish (9 cm) lined by filter paper (Whatman 100) and were directly sprayed with 3 ml conidial suspension using an atomizer.

The control insects were sprayed with 0.02% Tween 80[R] in sterile distilled water only. After air drying the treated termites they were carefully transferred to Petri-dishes (9 cm) containing fine sieved (2 mm) and sterilized soil. Small pieces of fresh fungal combs and a few soft wood pieces were provided as food. The treated insects were maintained at 25 ± 2°C and 96–100% r.h. Insects (100) were used for each [illegible]atment and there were four replications. Ob[illegible]ations on the mortality of termites were recorded at every 12 hr upto 7 days. To confirm the pathogenicity of the fungi the entomopathogenic fungi were isolated from the infected termites and fresh healthy termites were reinoculated.

Bioassay

Bioassay tests were carried out with five isolates of *B. bassiana* and one *M. anisopliae* strain obtained from different locations (Table 1). A range of conidial concentrations from 10^4 through 10^8 conidia/ml of each of the fungal pathogen isolate were prepared and termites were treated as indicated in screening assays. Observations on the mortality were recorded at 6 hr interval for 7 days. The assay procedure was followed for all the three morphogenetic forms, namely workers major, workers minor and the soldier caste.

Statistical analysis

The mortality data were transformed to Arcsin percentage (for percentage data) or square root values for the purpose of statistical analysis, and per cent mortalities were corrected as suggested by Abbott (1925). The treatment means were separated by Duncan's multiple range test (DMRT). The data collected on dosage-mortality and time-mortality responses was subjected to "Probit analysis" (Finney, 1964).

RESULTS AND DISCUSSION

Among the fungal pathogens tested against *O. obesus*, all of the *B. bassiana* and *M. anisopliae*

Table 2. Pathogenicity of fungal pathogens against *Odontotermes obesus*

Fungus	Per cent corrected mortality after seven days[a]		
	Workers major	Workers minor	Soldiers
Metarhizium anisopliae (Ma)	43.67d	47.33d	33.67cd
M. flavoviride	8.00e	13.33e	5.00e
Paecilomyces lilacinus	6.33e	8.00f	3.33e
P. fumosoroseus	2.00f	2.00g	1.00f
B. bassiana (BNG)	58.33c	57.67c	41.67bc
B. bassiana (NDL)	37.33d	44.67d	28.00d
B. bassiana (BPT)	76.67a	80.67a	51.00a
B. bassiana (CBE)	45.00d	52.33cd	36.33c
B. bassiana (PHP)	68.67b	70.33b	44.67ab

[a] Means separation by DMRT at 5% level for each termite caste.
PHP = Phillipines isolate.
NDL = New Delhi isolate.
BNG = Bangalore isolate.
CBE = Coimbatore isolate.
BPT = Bapatla isolate.

isolates were virulent, affecting significantly higher mortalities than *M. flavoviride*, *P. lilach*[illegible]nd *P. fumosoroseus*, which were only slightly pathogenic. Among the five isolates of *B. bassiana* viz. CBE, NDL, PHP, BPT and BNG, the strain BPT isolated for Andhra Pradesh caused highest mortality among all the three morphogenetic forms of termites (Table 2). Workers minor were most susceptible to all the pathogens tested followed by workers major. The soldiers were the least affected by fungal pathogens (Table 2). Pathogens such as *Verticillium lecanii*, *Paecilomyces farinosus* and *Nomuraea rileyi* were not pathogenic as they neither caused mortality nor any kind of abnormality.

The probit analysis of the dosage-mortality response and time-mortality response was carried out on the three morphogenetic forms of *O. obesus* with the five isolates of *B. bassiana* and one isolate of *M. anisopliae*. Results indicated that *B. bassiana* isolate BPT was the most effective recording the lowest LC_{50} and LT_{50} values followed by isolates PHP, BNG, CBE, MA and NDL, respectively. It further confirmed that workers minor was the most susceptible termite caste followed by workers major and soldiers, respectively (Tables 3 an[illegible]

The variation in virulence among the different isolates within the single species viz. *Beauveria bassiana* may be due to heterokaryosis, somatic recombination and saprobic growth of the fungus in the environment prior to its interaction with the insects (Roberts and Yendol, 1971). *Metarhizium anisopliae* is one of the most frequent fungal pathogens of soil insects. It attacks larvae of Tipulidae, Elateridae, Carabidae and Scarabaeidae of the genera *Hoplia*, *Amphimallon* and *Melolontha*. Epizootics have, however, been found affecting only wire worms (*Agriotes* spp.) and larvae of *Amphimallon solstitialis*. This variation in infection may be due to the presence of different pathotypes (strains) with different host preference (specificity) (Ferron et al., 1972; Fargues, 1976). It is well established, now, that entomopathogenic fungi have a certain specificity (Fargues and Remaudiere, 1977). In the same species of fungus, different strains can have very different

Table 3. Susceptibility of *Odontotermes obesus* to different fungal isolates

Fungus	Termite caste	$\chi^{2\circ}$ (3)	LC_{50} (Conidia/ml) x 10^4	Fiducial limits (95%) x 10^4
B. bassiana (BNG)	MA	2.09	52.89	39.38–71.01
	MI	2.09	36.90	22.92–59.41
	SO	0.24	550.43	377.79–801.97
B. bassiana (NDL)	MA	1.75	330.63	222.69–490.87
	MI	1.48	221.12	144.18–339.11
	SO	3.36	949.72	605.39–1489.89
B. bassiana (BPT)	MA	0.50	9.98	7.86–13.54
	MI	1.93	5.65	3.55–9.00
	SO	2.06	52.50	32.48–84.86
B. bassiana (CBE)	MA	4.14	148.79	98.20–225.44
	MI	[illegible]	114.91	79.29–166.54
	SO	2.72	690.77	446.57–1068.50
B. bassiana (PHP)	MA	1.28	36.46	27.85–47.73
	MI	1.38	12.98	8.66–19.47
	SO	1.32	287.44	173.18–477.11
M. anisopliae (MA)	MA	1.05	225.32	160.21–316.89
	MI	2.08	148.67	101.17–218.48
	SO	0.40	780.38	502.17–1212.71

° All lines are significantly a good fit ($P < 0.05$).
MA = Workers Major.
MI = Workers Minor.
SO = Soldiers.
PHP = Philipines isolate.
NDL = New Delhi isolate.
BNG = Bangalore isolate.
CBE = Coimbatore isolate.
BPT = Bapatla isolate.

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49.29–166.54
[illegible]
27.85–47.73
8.66–19.47
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Table 4. Time-mortality response of *Odontotermes obesus* to different fungal isolates treated at concentration of 4 x 10^7 conidia/ml

Fungus	Termite castes	χ^2 (3)	LC_{50} (days)	Fiducial limits (95%) days
B. bassiana (BNG)	MA	1.16	3.86	3.69–4.03
	MI	1.38	3.77	3.66–3.88
	SO	1.17	4.56	4.38–4.74
B. bassiana (NDL)	MA	1.62	4.62	4.46–4.78
	MI	1.93	4.45	4.30–4.61
	SO	1.22	5.89	5.65–6.15
B. bassiana (BPT)	MA	1.58	3.49	3.37–3.61
	MI	0.64	3.26	3.13–3.40
	SO	0.71	4.02	3.87–4.17
B. bassiana (CBE)	MA	1.66	4.23	4.10–4.36
	MI	1.53	3.40	3.75–4.25
	SO	0.80	4.94	4.78–5.08
B. bassiana (PHP)	MA	1.27	3.83	3.60–4.07
	MI	1.64	3.66	3.52–3.81
	SO	1.17	4.37	4.23–4.52
M. anisopliae (MA)	MA	2.13	4.50	4.36–4.63
	MI	1.28	4.05	3.91–4.21
	SO	0.68	5.83	5.16–5.52

PHP = Phillipines isolate.
NDL = New Delhi isolate.
BNG = Bangalore isolate.
CBE = Coimbatore isolate.
BPT = Bapatla isolate.

activity spectra (Ferron et al., 1972). Study of this specificity by Fargues et al. (1976) showed the importance of mechanisms acting at the integument and in the body cavity.

Silturn and Bevzenko (1972) found variation in toxin production in different strains of *B. bassiana*, which could be correlated with the variation in virulence. Variation in the susceptibility of noctuid larvae to different geographical isolates of *Nomuraea rileyi* was observed by Ignoffo et al. (1976). Ferron (1978) found obvious differences in virulence between numerous strains of *B. brongniartii* against *Melolontha melolontha* L. and *Acanthoscelides obtectus* (Say). Field data suggest that strains of the same fungus exist, which are pathogenic only to adults of *M. melolontha* and others which attack only the larvae (Keller, 1986). In nature, living organisms such as the mycopathogens, undergo selection, recombination and mutation depending upon their ecological situation. This influences the genetic make up of the fungi which in turn can affect their virulence for a particular insect host (Ignoffo and Garcia, 1985).

There was variation in the relative susceptibility of the different developmental stages of *O. obesus*. The variation in the mortalities is a reflection of the progressive development of the entomopathogenic fungi over time. This phenomenon has been well documented by Getzin (1961), Gardner and Noblet (1978), Ignoffo et al. (1978) and Fargues and Rodriguez-Rueda (1980).

Variation in the susceptibility of the different forms of *O. obesus* may be as a result of cuticular or haemocoelic differences between the castes. Wood and Gruia (1984) reported that the amino acid composition of the larval cuticle varied between instars and discussed the possible influence of amino acid combination, certain amines and peptides on the infectivity of fungal pathogens. Host-pathogen interaction also occur in the haemocoel where many intrinsic factors operate. The chemical constituents of an insect also vary as it ages (Boman, 1981).

Mechanism of pathogenicity and specificity appears to be complex and successful fungal penetration into the host can be said to depend upon at least three factors, the abilities of the spore to adhere to the cuticle, to germinate and to penetrate enzymatically (Fargues and Remaudiere, 1977; Hall and Papierok, 1982). Evidently, there are many factors at the level of the cuticle and haemolymph which

may determine host specificity of fungi. A high degree of host specificity is observed in some species or strains such as *M. anisopliae* (Fargues, 1981) and *B. bassiana* (Kmitowa et al., 1977). Much more effort is needed on the subjects of infection mechanisms and host defences to elucidate the reasons for host specificity (Hall and Papierok, 1982).

Entomopathogenic fungi viz. *B. bassiana* and *M. anisopliae* have been used, as biocontrol agents, with varying degree of success, against soil dwelling insects and termites (Keller and Zimmermann, 1989; Hanel, 1981, 1982). Hanel and Watson (1983) reported heavy mortalities in the laboratory colonies of *Nasutitermes exitiosus* due to *M. anisopliae* and the disease persisted for at least 15 weeks. Further, the temperature (25–30°C) and 92–96% r.h. within the mounds of *O. obesus* does not show any circadian or seasonal fluctuations (Agarwal, 1975). Thus, these two abiotic factors are in a range most suitable for maximum sporulation and conidial germination of these mycopathogens (Walstad et al., 1970; Hussey and Tinsley, 1981).

Thus, in the light of the present study and findings of Hanel and Watson (1983) one gets a strong feeling that these mound building termites could be controlled using these fungi. However, there is need for further studies on the selection of fungal strains that can cope up with conditions in termite mounds. Similarly, further investigations are needed on the standardization of the dose, method of application, etc.

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